

**Study 3.18\* - Letter Report Number 6: Effect of Insect Repellent (DEET) on TSP Protection Against HD Challenge as Demonstrated by Lesion Area in Rabbits.**

**Task No:** Task 93-34

**Volume and Page:** Vol. 2.18, page 153 [Ref. 5.4.34]

**Conducting Laboratory and Location:** Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9050; Study No. SC940112]

**Date of Study Initiation:** February 6, 1996

**GLP Compliance:** No

**QA Report:** Yes ( ) No (x)

**Methods:** Per *in vivo* Protocol MREF 107: In Study A, sites were either left untreated or treated with 50  $\mu$ l DEET (N,N-diethyl-m-toluamide) insect repellent approximately 3 hours prior to ICD 2289 application. In Study B, the insect repellent was either applied and wiped off after approximately 3 hour wear period, or applied and left undisturbed for the minimal time required to pretreat other test sites (~12 min), followed by application of ICD 2289. In both studies, the 1  $\mu$ l HD challenge dose was administered topically approximately 1 hour after TSP application.

**Species/Strain:** New Zealand White Rabbits

**#/group or time point:** 8 animals/group, eight sites/animal

**Age, sex, weight:** 2-4 kg

**Drug Lot #, Radiolabel, and % Purity:** ICD 2289 - Lot no. 305 ~ 794; USAMRMC insect repellent lot no. IXDM.

**Observations and Times:** Following a 4 hour exposure period, all test sites were decontaminated and lesion scores calculated at 24 hours.

ANOVA and pairwise comparisons were performed using SAS.

**Results (Table 3.18.1):** In both studies, the mean LAR for a 1  $\mu$ l dose of HD was significantly ( $p < 0.05$ ) decreased for the ICD 2289 protected sites (LAR = 27%) compared to untreated. In Study A, application of insect repellent 3 hours prior to TSP application resulted in a mean LAR of 92%, indicative of compromised protective effect.

Table 3.18.1: Effect on TSP efficacy following DEET insect repellent application prior to application of ICD 2289 and subsequent exposure to 1  $\mu$ l HD.

Pretreatment Agents	n	Test Conditions	DEET Removal Method	LAR (%)	
				Arithmetic Mean (95% C.I.)	Geometric Mean (95% C.I.)
None	8	-	-	100	
ICD 2289	32	-	-	27 (11, 43)	26 (15, 46)
DEET + ICD 2289	32	a	none	91 (16, 166)	82 (41, 166)
DEET + ICD 2289	8	b	none	92 (70, 114)	77 (59, 102)
DEET + ICD 2289	8	c	moist towelette	122 (85, 158)	97 (72, 132)
DEET + ICD 2289	8	c	dry gauze	48 (32, 64)	39 (29, 52)

- a) DEET was applied 3 hours prior to TSP applications;  
 b) DEET was applied approximately 12 minutes prior to TSP applications  
 c) DEET was applied for 3 hours then removed just prior to TSP applications by either a moist (alcohol-free, dilute soap solution) towelette or a dry gauze pad.

Similar results were observed in Study B where the mean LAR for ICD 2289 protected sites was 17% and application of insect repellent for as little as 12 minutes or following removal of the insect repellent increased the LAR to 92%. Furthermore, removal of the DEET insect repellent with a moist towelette resulted in significantly higher LARs than removal with a dry gauze pad.

This and the preceding study demonstrate that the use of the standard issue insect repellent containing DEET, significantly reduced the antipenetration effect of ICD 2289, regardless of whether the repellent was applied before or after TSP application.

**Study 3.19\* - Letter Report Number 9: Effect of Insect Repellent (DEET) on TSP Protection Against VX Challenge as Demonstrated by RBC AChE Inhibition and Lethality.**

**Task No:** Task 93-34

**Volume and Page:** Vol. 2.19, page 308 [Ref. 5.4.45]

**Conducting Laboratory and Location:** Medical Research Evaluation Facility, Battelle, 505 King Ave., Columbus, OH [Contract DAMD 17-89-C-9050; Study No. G15534C]

**Date of Study Initiation:** January 7, 1997

**GLP Compliance:** ?

**QA Report:** Yes ( ) No (x)

**Methods:** Per *in vivo* Protocol MREF 107: This study was performed to determine whether insect repellent containing DEET would interfere with protection from VX if applied prior to the TSP. A 0.5 mg/kg challenge dose of VX was applied on clipped rabbit backs that had been either left untreated or pretreated with a regimen of DEET repellent followed by ICD 2289 or with TSP and VX. The insect repellent regimen consisted of 2 applications at the rate of approximately  $1.9 \mu\text{l}/\text{cm}^2$ , administered within the dose site on the day prior to and approximately 3 hours prior to pretreatment with TSP and 4 hours prior to dosing with VX. Test sites were decontaminated after 4 hours exposure to VX. Endpoints consisted of (1) RBC AChE activity relative to predose levels and (2) lethality.

**Species/Strain:** New Zealand White Rabbits

**#/group or time point:** 8 animals/group, eight sites/animal

**Age, sex, weight:** 2-4 kg

**Drug Lot #, Radiolabel, and % Purity:** ICD 2289 - Lot no. 305 — 794; USAMRMC insect repellent lot no. IXDM.

**Observations and Times:** Blood samples were collected from the medial ear artery at approximately -65, -35 and -5 minutes prior to VX dosing, and at approximately 1, 2, 3 and 4 hours after VX application.

ANOVA and pairwise comparisons were performed using SAS. Lethality rates were compared using Fisher's exact test at the 5% significance level.

**Results:** The results with and without ICD 2289 were consistent with previous studies. Application of insect repellent containing DEET prior to topical applications of VX had no protective effect. When applied prior to ICD 2289 application, it significantly reduced the effectiveness of ICD 2289 as an antipenetration agent, but did not completely neutralize its effect. In terms of both AChE activity (Table 3.19.1 and 3.19.2) and lethality (Table 3.19.3), insect repellent containing DEET reduced the effectiveness by approximately 50%.

Table 3.19.1: Effect on TSP efficacy following application of insect repellent containing DEET prior to application of ICD 2289 and subsequent exposure to 0.5 mg/kg VX.

Time (h)	Unprotected		DEET		ICD 2289		DEET + ICD 2289	
	AChE	%	AChE	%	AChE	%	AChE	%
0	2.3 ± 0.4	-	2.2 ± 0.4	-	2.3 ± 0.4	-	2.3 ± 0.4	-
1.0	0.0 ± 0.0	0 ± 0	0.1 ± 0.2	10 ± 10	1.6 ± 0.7	70 ± 30	1.5 ± 0.8	60 ± 30
2.0	0.0 ± 0.1	0 ± 0	0.0 ± 0.1	0 ± 0	1.5 ± 0.8	70 ± 30	0.9 ± 0.8	40 ± 40
3.0	0.0 ± 0.0	0 ± 0	0.0 ± 0.0	0 ± 0	1.4 ± 0.8	60 ± 30	0.6 ± 0.8	30 ± 30
4.0	0.0 ± 0.0	0 ± 0	0.0 ± 0.0	0 ± 0	1.2 ± 0.8	50 ± 30	0.4 ± 0.8	20 ± 30

Arithmetic mean ± sem

Table 3.19.2: Number of animals with &lt;50%, &lt;10% and 0% AChE activity at 4 hours.

AChE (%)	Unprotected	DEET	ICD 2289	DEET + ICD 2289
<50	23/23 (100%)	24/24 (100%)	8/23 (35%)	18/24 (75%)
<10	23/23 (100%)	24/24 (100%)	7/23 (30%)	15/24 (63%)
0	23/23 (100%)	24/24 (100%)	3/23 (13%)	10/24 (42%)

Table 3.19.3: Lethality rates after topical application of 0.5 mg/kg VX with or without ICD 2289.

Time (h)	Unprotected	DEET	ICD 2289	DEET + ICD 2289
4	23/23 (100%)	24/24 (100%)	3/23 (13%)	10/24 (42%)
8	23/23 (100%)	24/24 (100%)	5/23 (22%)	11/24 (46%)
12	23/23 (100%)	24/24 (100%)	5/23 (22%)	11/24 (46%)
24	23/23 (100%)	24/24 (100%)	6/23 (26%)	12/24 (50%)

*Review Note: Although the use of insect repellent containing DEET significantly reduced the protective effects of ICD 2289, some protective effect was still achieved, especially against the nerve agent VX. Optimally these products should not be used concurrently, and soldiers should be instructed not to apply insect repellent either prior to or following TSP application. However, if a repellent has already been applied, some limited benefit may still be derived from TSP use. Soldiers should be instructed to remove as much of the repellent as possible with a dry cloth prior to application of TSP.*

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## OVERALL SUMMARY OF NONCLINICAL EFFICACY

### 1. Summary of *in vitro* Efficacy Models

In studies utilizing M8 Chemical Agent Detector Paper, designed to detect chemical warfare agents by changing color upon contact with a variety of CWAs, pretreatment with ICD 2289 1 hour prior to challenge with TGD and HD was shown to effectively act as an antipenetration barrier for up to 6 hours.

CWA	w/TSP	w/o TSP
HD	>6 hours	Immediate
TGD	>6 hours	Immediate

Concurrent applications of military issue sunscreens did not appear to interfere with the *in vitro* effectiveness of TSP to block detection of HD. Concurrent applications of 6.25  $\mu\text{g}/\text{cm}^2$  permethrin (in isopropanol) also did not appear to interfere with the antipenetration effects of ICD 2289 when challenged with HD. However, when tested at 62.5  $\mu\text{g}/\text{cm}^2$ , two (2/9) breakthroughs occurred within the first hour after HD challenge. The dose response suggests that permethrin may have altered the antipenetrant effects of ICD 2289.

The time interval between application of TSP, i.e., wear-time, and subsequent challenge with HD was also evaluated for up to 24 hours. Time intervals of 5 minutes and 24 hours between application of TSP and HD did not appear to have any adverse effect on the *in vitro* performance ICD 2289.

### 2. Summary of Nonclinical *in vivo* Efficacy Studies

***In Vivo Efficacy in Animals Models:*** Multiple biological endpoints were used to evaluate the effectiveness of TSP following topical exposures of HD in guinea pigs and HD, GD, TGD, VX and T-2 in rabbits. Endpoints evaluated included measurements of RBC and plasma AChE activity and lethality for the nerve agents, and dermal irritation for HD and T-2.

The 0.5 mg/kg dose of VX represents 10 times the 24-hr LD<sub>50</sub> dose for unprotected rabbits and the 3.35 mg/kg TGD dose represents the historical 24-hr LD<sub>50</sub>.

***Antipenetration effects of ICD 2289 on Dermal Toxicants:*** In rabbits, applications of ICD 2289 prior to a 4-hour challenge with 1  $\mu\text{l}$  liquid HD significantly reduced both the size of the lesions and the mean lesion area ratio (LAR) when compared to pretreatment with PEG 540. Arithmetic mean lesion sizes at 24 hours were 3.5 to 5 fold smaller than lesions at unprotected sites and the arithmetic mean LAR was reduced by 70 to 80 % (Studies 2.6, 2.8 2.9 and 2.14). Pretreatment with ICD 2289 prior to a 3-hour challenge with HD vapor (1.4 mg/L) in guinea pigs, was also shown to significantly reduce erythema by 43% (Study 2.7) over a 4 hour treatment/observation period.

Pretreatment with ICD 2289 was also shown to effectively block all macroscopic signs of dermal irritation 24 to 48 hours following a 6-hour challenge with T-2 toxin (Study 2.15). Histopathological

findings were also significantly ( $p < 0.05$ ) diminished from moderate to marked lesions in controls to only minimal when present at ICD 2289 protected sites. Histological lesions from the TSP pretreated sites consisted of superficial dermatitis, edema, folliculitis, and epidermal necrosis; and intradermal pustules in 1/8 sites. Unprotected sites also presented with moderate to marked deep dermatitis, panniculitis, dermal necrosis and intradermal pustules at 8/8 sites.

Following a 15 minute challenge of 0.1 ml of 1.0% CS/TOF with and without TSP, signs of erythema were very mild and first appeared 15-60 minutes after exposure. Edema was observed only after 24 hours. Test sites protected by ICD 2289 demonstrated significantly milder reactions (barely perceptible) when compared to reactions at unprotected sites (Study 2.16). At 4 and 24 hours post challenge, all unprotected sites (20/20) presented with erythema and edema whereas only mild erythema was observed in 6/20 animals in the ICD 2289 treatment group at 4 hours and 9/20 animals (1 with edema) at 24 hours.

#### *Antipenetration effects of ICD 2289 on Cholinesterase Inhibitors:*

In terms of mean absolute and relative RBC AChE, ICD 2289 demonstrated significant protection relative to the unprotected animals against 4-hour challenges with both 3.35 mg/kg TGD and 0.50 mg/kg VX (See Table). However, pretreatment with ICD 2289 failed to prevent the loss of >50% RBC AChE activity in 35% of the animals challenged with TGD and 46% of the animals challenged with VX. In general, there was no observable recovery of RBC AChE activity at 24 hours in surviving animals demonstrating significant reductions at earlier time points and a few animals which were above 50% RBC AChE activity at the earlier time points, were below 50% activity or dead at 24 hours.

Effects of CWAs on RBC AChE Activity and Mortality (Study 3.12).

Parameter	Time (h)	TGD		VX	
		w/o TSP	ICD 2289	w/o TSP	ICD 2289
Mean AChE Levels (U/ml)	4	0.2 ± 0.2	1.2 ± 0.4	0.0 ± 0.0	1.1 ± 0.8
Mean AChE Activity (%)	4	10 ± 10	60 ± 20	0 ± 0	50 ± 40
% Animals w/100% AChE Activity	4	0 %	0 %	0 %	8 %
% Animals w/<50% AChE Activity	4	100 %	35 %	100 %	46 %
Mortality Rate	4	8 %	4 %	100 %	4 %
	24	12 %	8 %	100 %	8 %

In terms of mean absolute and relative whole blood AChE, ICD 2289 demonstrated significant protection relative to the unprotected animals against 4-hour challenges with both 8.9 mg/kg GD and 0.50 mg/kg VX (See Table). However 4-hour exposures to both GD and VX resulted in 75% and 54% of the animals, respectively, losing greater than 50% of their whole blood AChE activity. It should be note here, that exposures to GD resulted in some reduction in whole blood AChE activity in over 99% of the animals (145/146), whereas exposures to VX resulted in reductions in whole blood AChE activity in only 67% of the animals (96/144), with approximately 2/3 of these animals dying, while 1/3 of the ICD 2289 pretreated animals maintaining 100% activity over the 4

hour challenge period. It appears that with VX, any compromise of the antipenetration barrier resulted in significant exposures.

Effects of CWAs on Whole Blood AChE Activity and Mortality (Study 3.13).

Parameter	Time (h)	GD		VX	
		w/o TSP	ICD 2289	w/o TSP	ICD 2289
Mean AChE Levels (U/ml)	4	0.0 ± 0.1	0.4 ± 0.3	0 ± 0	0.6 ± 0.6
Mean AChE Activity (%)	4	0	30 ± 20 %	0	50 ± 40 %
% Animals w/100% AChE Activity	4	0	<1.0 %	0	33 %
% Animals w/<50% AChE Activity	4	100 %	75 %	100 %	54 %
Mortality Rate	4	100 %	7 %	100 %	35 %
	24	100 %	9 %	100 %	39 %

In terms of lethality, ICD 2289 was effective in significantly reducing the lethal effects of VX by protecting over 90% of the animals from death in comparison to the 100% mortality observed within 4 hours in the unprotected animals. There was no significant difference in mortality between animals with and without ICD 2289 pretreatment and challenge with TGD.

In terms of lethality, ICD 2289 was effective in significantly reducing the lethal effects of GD by over 90% at 24 hours post challenge. Studies 2.12 and 2.13 demonstrated mortality rates of 8% and 39%, respectively, resulting in a reduction of lethal effects by 50-90% compared to controls. There was no significant difference in mortality between animals with and without ICD 2289 pretreatment and challenge with TGD.

*Review Note: Although there was significant mortality observed in rabbits pretreated with TSP prior to challenge with VX, it should be noted that this dose is 10 fold greater than the LD<sub>50</sub> dose in rabbits and expected to be significantly greater than exposures in the field. In humans, the percutaneous LD<sub>50</sub> for GD is estimated to be 100 mg (or approximately 1.67 mg/kg).*

#### Summary of the Effect of Pesticides and Sun Screen on TSP

There were notable decreases observed in the protective effects of ICD 2289 to 1 µl HD challenge when some types of camouflage paints were applied post-TSP application. Applications of loam camouflage paint increased the LAR by 300 % and sand camouflage paint increased the LAR by 39% when compared to ICD 2289 alone. However, these increases may be somewhat inflated due to low lesion scores for ICD-2289 when used alone. There were no effects observed on ICD-2289 efficacy with either bright green or white camouflage paints (Study 2.17).

The use of insect repellent containing DEET applied either prior or post-TSP application significantly reduced the effectiveness of ICD 2289 when challenged with 1 µl HD. Applications of insect repellent containing DEET following application of ICD 2289 resulted in a 400% increase in

the LAR when compared to TSP alone (Study 2.17). When compared to sites pretreated with ICD 2289 alone, applications of insect repellent containing DEET 3 minutes or 3 hours prior to the TSP resulted in 3-5 fold increases in the LAR. Removal of the insect repellent with a moist towelette 3 hours prior to application of TSP resulted in 5-7 fold increases in LAR when compared to site protected only with ICD 2289, whereas removal of insect repellent 3 hours prior to application of ICD 2289 resulted in only ~ 50% reduction in TSP efficacy (Study 2.18). The effect of removal of insect repellent just prior to application of TSP was not studied.

Similar effects were observed in challenge tests with VX, insect repellent containing DEET applied prior to ICD 2289 application significantly reduced the effectiveness of ICD 2289 but did not completely neutralize its effect. In terms of both AChE activity and lethality, insect repellent containing DEET reduced the effectiveness of ICD 2289 by approximately 50%. However, lethality was still reduced by ~50% when comparing animals treated with both insect repellent and ICD 2289 to unprotected animals in which 100% mortality was observed within the challenge period.

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**SECTION IV: TOXICOLOGY STUDIES**

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**1. Safety Evaluation of TSP ICD-2289**

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**Study 4.1: Acute Oral Toxicity and Metabolism of the Topical Skin Protectant Formulation ICD 2289 in Rats.****Study No:** 75-51-2513-96**Volume and Page No.:** Vol. 2.19, page 018 [Ref. 5.4.39]**Conducting Laboratory and Location:** Department of the Army, U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD**Date of Study Initiation:** March 1995**GLP Compliance:** Yes**QA Report:** Yes ( x ) No ( )**Methods and Dosing:** This study was performed to determine the acute oral toxicity and metabolism of the topical skin protectant formulation ICD-2289. Rats were treated with a single oral dose of ICD 2289 by gavage.**Species/Strain:** Sprague-Dawley Rats**#/sex/group or time point:** 8/sex (1/sex/dose)**Age:** ?**Weight:** Males - 198-243 g; Females - 215-319 g**Supplier:** \_\_\_\_\_**Satellite Groups Used for Toxicokinetics or Recovery:** Laboratory evaluations were performed in 15 male rats receiving a single oral dose of ICD 2289 at the determined NOAEL dose and compared to 15 control (untreated) rats.**Dosage Groups in Administered Units:** Nominal doses were 300, 439, 658, 987, 1480, 2222, 3333, and 5000 mg/kg. Actual doses ranged from 41 to 3240 mg/kg in males and from 201 to 3311 mg/kg in females. Control rats were untreated.**Route, Form, Volume and/or Infusion Rate:** Neat mg/kg doses (estimated = 2.05 gm/cc)**Drug Lot #, Radiolabel, and % Purity:** Lot No. 329 ~ 794**Formulation/Vehicle:** Neat**Observations and Times:****Clinical Signs:** Animals were observed daily through 14 days.**Body Weights:** Animals were weighed just prior to treatment and again on days 1 (24-hrs post dosing), 3, 7 and 14.**Laboratory Evaluations:** Blood and urine samples were collected from 5 male rats/time point/satellite group at 24, 48 and 72 hours.**Gross Pathology:** Animals were euthanized on day 14 and necropsied. Male satellite animals were also necropsied and gross pathological observations were recorded.**Organs Weighed:** The adrenals, brain, liver, kidneys, gonads, and spleen were weighed and organ to body weight ratios were computed for the male satellite animals.**Results:****Acute Toxicity:** Male and female rats receiving a single dose of ICD 2289 were not affected at the highest levels tested, i.e., 3240 mg/kg and 3311 mg/kg, respectively.



**Satellite Study:** No toxic signs were observed in male rats through 72 hours following a single oral dose of ICD 2289 at a nominal dose of 3,240 mg/kg. Absolute doses averaged  $3189 \pm 440$  mg/kg. There were no significant differences in hematology, clinical chemistry and urinalysis parameters, or in organ weights at 24, 48 or 72 hours post-dosing in male animals when compared to untreated animals.

**Key Study Findings:** The NOAEL following an acute oral dose in rats was approximately 3240 mg/kg ( $19,440 \text{ mg/m}^2$ ). The equivalent dose in man would be approximately 540 mg/kg ( $19,980 \text{ mg/m}^2$ ) or approximately 32 g for an average adult weighing 60 kg. Accidental ingestion of this magnitude is unlikely in humans.

**Study 4.2: Assessment of Dermal and Ocular Effects of Candidate Topical Skin Protectant Formulation ICD-2289.**

**Study No:** 75-51-Y1LD-94(B)

**Volume and Page No.:** Vol. 2.17, page 183 [Ref. 5.4.28]

**Conducting Laboratory and Location:** US Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD

**Date of Study Initiation:** January, 1994

**GLP Compliance:** Yes

**QA Report:** Yes (x) No ( )

**Methods and Dosing:** This study was conducted to determine the potential dermal and ocular hazards associated with the use of candidate TSP ICD 2289. Evaluation of primary skin irritation, photochemical skin irritation, and primary eye irritation was conducted in rabbits of either sex. Sensitization potential was evaluated in guinea pigs.

- 1) **Skin Irritation – Intact and Abraded Skin:** A single application of 0.5 g of ICD 2289 was applied to the clipped intact and abraded skin on the backs of six rabbits under a 2 x 2 inch gauze pad and overwrapped with an occlusive ——— dressing for 24 hours. After 24 hours, dressings were removed and excess material gently removed. Test sites were evaluated for irritation at 1, 48 and 72 hours and at 7 days. Sites were scored according to the Draize method.
- 2) **Eye Irritation:** A single 0.1 gm dose of the ICD 2289 was administered to the conjunctival sac of each of six rabbits. Eyes were examined for gross signs of irritation relative to the untreated eye at 24, 48 and 72 hours and 7 days following exposure. Eyes were scored according to the Draize method.
- 3) **Photochemical Skin Irritation:** Applications of 0.05 g ICD 2289 are applied to the right side of the clipped backs of 6 rabbits. After 5 minutes, the backs were exposed to UV light (365 mm) for ~10 minutes. Following UV exposure of the rabbits, the left side of the same animal was treated with identically to the right side, except it was not irradiated. Oil of Bergamot (0.05 ml of a 10% ethyl alcohol solution) was used as the positive control. Sites were scored according to the Draize method at 24, 48 and 72 hours post-exposure. A photochemical response was considered positive when the net erythema score was greater than 1.0 and/or the edema score was 0.5 or greater.

- 4) **Skin Sensitization:** Test method was based on the method of Buehler. ICD 2289 (0.1 g) was applied under \_\_\_\_\_ patches to the clipped backs of 20 guinea pigs 6 hours, once per week for 3 weeks. Following a 2-week rest period the animals were challenged with a single application of 0.1 g ICD 2289. Sites were evaluated 24 and 48 hours after challenge and compared to naïve animals.

**Animals:**

Assay -	Dermal Irritation	Ocular Irritation	Phototoxicity	Sensitization
Species/Strain:	NZ White Rabbits	NZ White Rabbits	NZ White Rabbits	Hartley-Albino GP
#/sex/group:	6	6		20
Age:	?	?	?	?
Weight:	3.5-4.8 kg	3.5-4.8 kg	3.5-4.8 kg	350-475 g
Supplier:				
Dosage Units:	500 mg	100 mg	50 mg	100 mg

**Drug Lot #, Radiolabel, and % Purity:** UNI73.93

**Results:**

- 1) **Skin Irritation – Intact and Abraded Skin:** Only 1/6 animals evinced barely perceptible erythema at 72 hours post-dosing on intact skin. There were no gross signs of erythema on abraded skin or signs of edema on either intact or abraded skin. Under the conditions of this assay, ICD 2289 was not considered a primary skin irritant.
- 2) **Eye Irritation:** At no evaluation time point were there signs of ICD 2289 –induced gross signs of irritation to the cornea, iris or conjunctiva. Under the conditions of this assay, ICD 2289 was not considered a primary ocular irritant.
- 3) **Photochemical Skin Irritation:** At no evaluation time point were there signs of ICD 2289 – induced photochemical skin irritation. Under the conditions of this assay, ICD 2289 was not considered to potentiate photo-induced irritation.
- 4) **Skin Sensitization:** There was no sign of dermal irritation at ICD 2289 challenge sites in any of the animals tested. Under the conditions of this assay, ICD 2289 is not considered to be a potential sensitizer.

**Key Study Findings:** ICD 2289 did not produce primary skin irritation, ocular injury or photochemical irritation in rabbits. No skin sensitization was observed in guinea pigs.

**Study 4.3: Dermal Toxicity of the Topical Skin Protectant Formulation ICD-2289 in Rabbits through 21 Days.**

**Study No:** 75-51-Y3HX-95 (Administrative No. 85-48-2332)

**Volume and Page No.:** Vol. 1.17, page 217 [Ref. 5.4.29]

**Conducting Laboratory and Location:** U. S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD

**Date of Study Initiation:** July 1995

**GLP Compliance:** Yes

**QA Report:** Yes (x) No ( )

**Methods and Dosing:** The purpose of the study was to determine the effects in rabbits of repeated (daily) dermal applications of ICD 2289. ICD-2289 at a concentration of 2000 mg/kg was spread onto a 4x4 gauze pad. The pad was placed on the clipped backs of animals and secured with an occlusive elastic bandage, 6 hours/day, 5 days/week for 3 weeks. An equal number of animals served as controls and wore the gauze pad and overwrap.

**Species/Strain:** New Zealand White rabbits

**#/sex/group or time point:** 10/sex/group

**Age:** ?

**Weight:** Males - 2.5-3.0 kg; Females 2.4-2.7 kg

**Supplier:** \_\_\_\_\_

**Dosage Groups in Administered Units:** 0 and 2000 mg/kg ICD 2289

**Drug Lot #, Radiolabel, and % Purity:** Lot no. 329 ~194

**Formulation/Vehicle:** Neat

**Observations and Times:**

**Clinical Signs:** Animals were observed daily for clinical signs of toxicity.

**Body Weights:** Weights were recorded weekly.

**Hematology and Clinical Chemistry:** Blood specimens were collected pre-dosing and following the final dose. Hematological and serum chemistry (glucose, cholesterol, lactic dehydrogenase, total protein, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, alkaline phosphatase, creatinine phosphokinase and triglycerides) analyses were performed.

**Gross Pathology:** Twenty-four hours following the final treatment, animals were weighed and necropsied.

**Organs Weighed:** See Histology Table

**Histopathology:** Histological examinations performed on the following tissues:

Adrenals *	Heart w/aorta	Peripheral	Sternum
Brain *	Jejunum	Nerve	Stomach
Cecum	Kidneys *	Pituitary	Thymus
Colon	Liver *	Rectum	Thyroid/Parathyroid
Duodenum	Lungs	Salivary	Trachea
Esophagus	Lymph	Glands	Urinary
Femur	Mammary	Skin - Normal	Bladder
Gall Bladder	Muscle (Thigh)	Skin - Treated	
Gonads	Pancreas	Spinal Cord	
w/access *		Spleen *	

Histopathological evaluations were performed by \_\_\_\_\_

**Results:**

**Clinical Signs:** With the exception of intermittent diarrhea, occurring in one female early in the study, there were no clinical signs of toxicity in any of the treated animals. One control animal died on day 6 of mucoid enteropathy, which was not considered to be related to the testing procedure. No effects to skin surface (erythema, edema, etc) were noted in any of the animals receiving ICD-2289.

**Body Weights:** There were no statistically significant difference in body weight or body weight gains between treated and control animals.

**Hematology:** There were no statistically significant differences in pre- and post-dosing hematological values or between treated and controls for male animals. However, one male did have a significant WBC differential shift consisting of increased monocytic cells (~4 fold) offset by a decrease in lymphocytic cells (~7 fold). For females, post-dosing hematology results for females were lost, however, there were no significant differences in the differential blood counts for females.

**Clinical Chemistry:** There were statistically significant decreases in post-dosing mean BUN and total protein in treated females when compared to controls. However, all values were within the normal ranges and are not considered biologically relevant. There were no significant differences in treated male animals either between groups or between pre-and post-dosing data.

**Organ Weights:** Absolute and relative (body weight-adjusted) mean liver weights were decreased by approximately 15% in females. However, when corrected using brain weight-adjusted relative weights, there was no significant increase when compared to untreated controls. There were no other significant differences in either absolute or corrected mean organ weights for any other organs evaluated in males or females when compared to untreated controls.

**Gross Pathology:** At the time of necropsy, there were no observed gross lesions in any tissues examined.

**Histopathology:** Histopathological evaluation of treated skin revealed a higher incidence of subacute inflammation than observed in controls. The cutaneous lesions consisted of "superficial dermal infiltrations of lymphocytes, heterophils and macrophages that had no [discernable] pattern of distribution relative to dermal structures". According to the pathologist, the lesions were compatible with response to a mild irritant. The incidence and severity of subacute inflammation are presented in Table 1. No other significant, dose-related findings were reported.

Table 1: The incidence of subacute inflammation of the skin from rabbits treated dermally with 2000 mg/kg/day ICD-2289 for 6 hours/day, 5 days/week for 3 weeks.

	Males				Females			
	Control		ICD-2289		Control		ICD-2289	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
No. Examined	10	10	10	9	8	7	9	8
Normal	7	7	3	6	5	2	3	5
Inflammation:								
Trace	2	3	3	3	3	4	5	3
Mild	1	0	3	0	0	1	1	0
Moderate	0	0	1	0	0	0	0	0

Note: Treated samples were taken from clipped back; untreated skin specimens were taken from the same animal but from shoulder area. Control animals had occluding wraps, but no test substance.

**Key Study Findings:** Repeated applications of 2000 mg/kg/day (24.0 g/m<sup>2</sup>/day) ICD 2289 to the skin surface of rabbits did not cause any significant signs of systemic toxicity. Dermal signs of

toxicity consisted of mild, subacute inflammation. (*Note: Potential maximum human exposure = 51.8 g/m<sup>2</sup>*)

## 2. Safety Evaluation of Fomblin® HC/25

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### **Study 4.4: Safety Evaluation of Perfluoropolyethers, Liquid Polymers used in Barrier Creams and Other Skin-Care Products.**

**Reference:** Malinverno G, Pantini G and Bootman J (1996) Food and Chemical Toxicology 34:639-50

**Volume and Page No.:** Vol. 2.19, page 005 [Ref. 5.4.38]

**Conducting Laboratory and Location:** Studies were conducted by \_\_\_\_\_

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**GLP Compliance:** ?

#### Methods and Dosing:

**Acute Toxicity:** Acute toxicity was evaluated in CD rats (5/sex/group), by the oral, dermal and i.p. routes. The test solution was dosed without dilution at 15 g/kg (orally), and 5 g/kg (dermally and by ip). Dermal exposure was for a 24 hour period under occlusive dressing and animals were observed for 14 days after dosing.

**Primary Dermal Irritancy:** Primary skin irritancy was assessed in New Zealand White rabbits (n=6). Topical applications consisted of 0.5 ml applied to sites with intact and abraded skin for 24 hours under an occlusive dressing.

**Primary Ocular Irritancy:** Ocular applications consisted of 0.1 ml instilled into the conjunctival sac of the right eye of each rabbit (n=6). Reactions were assessed at intervals up to 72 hours or 7 days after dosing.

**Repeated Application Dermal Irritancy:** New Zealand White rabbits (6/sex) were treated with HC/25 or saline for 14 consecutive days on abraded and intact skin under a semi-occlusive dressing. Local reactions were recorded daily, and after termination all skin sites were subjected to histopathological examinations.

**Repeat Dose Toxicity:** Possible cumulative toxicity was investigated in subacute (4-wk) rat oral toxicity test. CD rats (5/sex/group) were dosed orally by gavage with 1000 mg/kg/day HD/25 or distilled water. Satellite groups (3/sex) were included for toxicokinetic evaluation. Evaluation parameters included the following: daily clinical evaluations; weekly evaluation of body weight and food consumption; hematology and clinical chemistry on day 25; urinalysis on samples collected between days 28 and 29; complete necropsies were performed including selected organ weights and all abnormal tissues were subjected to histopathological examination. Bile, blood and urine samples were obtained from the satellite animals.

**Skin Sensitization:** Sensitizing potential of HC/25 was tested in Dunkin-Hartley albino guinea pigs (10/sex) using the maximization test method of Magnusson and Kligman (1969). The induction doses were 2 x 0.05 ml with FCA and the challenge dose was 0.6 ml HC/25. Closed patch irritation studies were also performed on humans in Japan and Europe.

**Photosensitizing Potential:** Photosensitizing potential was also assessed in Dunkin-Hartley albino guinea pigs using maximization test procedures based on the methods of Morikawa (1974) and Maurer (1983). One day following intradermal injections of FCA, animals were given 11 separate 0.1 ml induction treatments followed 20 minutes later by UVA/UVB irradiation every second day over a period of 21 days. Challenge on day 35 comprised topical application of 0.05 ml HC/25.

**Comedogenicity:** HC/25 was evaluated for comedogenic action by repeated application to the epidermis of the rabbit ear following the method of Kligman and Kwong (1979). One ml of undiluted test material was applied daily to the ear of 6 male New Zealand White rabbits for 14 days. Local reactions were assessed visually each day, and at termination, epidermal strips were examined under a stereomicroscope.

**Mutagenicity Testing:** The bacterial mutation assay of Ames was employed following the EC test guideline (1992), using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. HC/25 diluted in DMSO was tested at concentrations in the range of 50 to 5000 µg/plate.

**Results:** Review of the individual animal and group findings are summarized below:

**Acute Toxicity:** Acute doses of 15 g/kg (orally), and 5 g/kg (dermally and by ip) did not produce any significant signs of toxicity in rats.

**Primary Dermal Irritancy:** There were no signs of skin irritancy in New Zealand White rabbits following 24 hour, occluded topical applications of 0.5 ml to either intact or abraded skin.

**Primary Ocular Irritancy:** There were no signs of ocular irritancy at either 72 hours or 7 days after dosing.

**Repeated Application Dermal Irritancy:** There were no macro- or microscopic signs of skin irritancy in New Zealand White rabbits following repeated applications on abraded and intact skin under a semi-occlusive dressing 14 consecutive days.

**Repeat Dose-Toxicity:** In the 4-week oral toxicity study, rats showed no significant changes in clinical behavior, body weight gains, food consumption, or clinical laboratory parameters. At autopsy, there were no consistent macroscopic abnormalities or changes in relative organ weights when compared to controls. The only apparent difference in test over control animals related to microscopic findings in the kidney tubules of male rats; 4/5 test males showed some basophilic and/or dilated tubules compared to 0/5 controls.

The absence of HC/25 (or degradation products) from circulating blood and bile is strongly indicative of non-absorption of HC/25.

**Skin Sensitization and Photosensitizing Potential:** HC/25 was wholly inactive in the skin sensitization tests with and without UV irradiation. Furthermore, there were no reports of human sensitization.

**Comedogenicity:** There were no signs of comedogenicity following repeat applications to rabbit ears.

**Mutagenicity Testing:** There was no indication of mutagenicity potential for HC/25.

**Study 4.5: Experimental Assessment of the toxicity of the Fluorine Oil "Fomblin Y 04."**

**Study No:** Translated Manuscript by A Starek, Z Chlap and D. Cembala in *Folia Medica Cracoviensis* 19(1):81-88, 1977

**Volume and Page No.:** Vol. 2.16, page 272 [Ref. 5.4.19]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** ?

**GLP Compliance:** ?

**QA Report:** Yes ( ) No ( x )

**Methods and Dosing:** Fomblin Y 04  $[-(O-CF(CF_3)-CF_2)_n-(O-CF_2)_m-]$  is similar in structure to Fomblin Y25. It was tested for general and local acute toxicity, local subacute toxicity, primary irritation effect on the skin and mucous membranes, and sensitizing properties in Wistar rats, guinea pigs, and rabbits.

- 1) In the first study, 25 fasted male rats were dosed by oral gavage to determine general acute ( $LD_{50}$ ) effects. The maximum dose administered was 20 ml/kg neat Fomblin. Animals were euthanized on days 4, 8 and 14 at which time blood samples were taken by cardiac puncture and specimens of the liver, kidneys, spleen, myocardium, brain, esophagus and stomach were taken for microscopic evaluation. Blood samples were evaluated for the following enzyme activity: AST, SDH, GD and alkaline phosphatase.
- 2) Hematology parameters were evaluated in a second group of 6 rats/sex dosed p.o. with 20 ml/kg neat Fomblin or 5 rats/sex dosed with 20 ml/kg 0.9% NaCl, blood samples were taken pre-dosing and on days 7 and 14 post-dosing from the tail vein.
- 3) The measurement of the acute and subacute toxicity after the application of Fomblin using the tail method of Massmann was performed on 5 male rats/group. Rats were treated with either neat Fomblin or 0.9% NaCl for 6 hours. In the absence of pronounced symptoms of intoxication at 24 hours, treatments were repeated daily for 10 consecutive days and animals were monitored for 3 weeks post-dosing. Blood samples were taken on days 1, 7, 14 and 21 post-dosing and all animals were subjected to gross and microscopic examinations at study termination.
- 4) The long-term toxicity of Fomblin was determined on 4 rats/sex. Animals were placed in exposure chambers and exposed to a mist of the test preparation in a concentration of  $\sim 1500$  mg/m<sup>3</sup> for 6 hours, 6 days/week for 4 weeks. Rats were weighed daily and observed for clinical signs of toxicity. Blood samples for hematologic evaluations and determination of

maleic dehydrogenase activity were collected prior to dosing and on days 7, 14 and 28 of exposure. Animals were examined microscopically with special attention to the lung.

- 5) The primary dermal and ocular irritating effect was determined on 4 rabbits by the method of Draize.
- 6) Sensitizing potential was determined on 6 guinea pigs by the method of Lansteiner and Jacobs.

### **Results:**

**Clinical Signs:** There were no deaths following p.o. administration of Fomblin Y 04. The LD<sub>50</sub> was determined to be greater than the highest dose tested of 20 ml/kg or 37.4 g/kg.

**Body Weights:** There were small time-dependent decreases in body weight: ~3% following the single oral dose and ~12% following 4 weeks of topical dosing.

**Local Effects:** Fomblin Y 04 demonstrated no local acute or subacute toxicity dermal toxicity in rats, primary dermal or ocular irritant action in rabbits, or sensitizing properties in guinea pigs.

**Hematology:** Small increases in hemoglobin (<10%) were noted in Fomblin treated animals. Decreases in WBC counts of 14 and 19% were also noted following repeat topical and inhalation administration, respectively.

**Clinical Chemistry:** There were no statistically significant changes in the chemistry parameters following the acute oral dose or repeated topical administration. However, following 4 weeks of dosing by inhalation, there were elevations in AST, ALT and maleic dehydrogenase activity.

**Gross Pathology:** There were no signs of macroscopic changes to internal organs found following either acute or subacute administration by either oral, topical or inhalation routes of exposure.

**Histopathology:** Following the acute dose the following nonspecific changes were observed: small degenerative changes in the liver, swelling of the parenchymatous membranes of the renal tubules and a rather large number of hyaline casts, hemosiderin deposits in the spleen, and inflammatory exudations in the stomach wall of some rats. Following repeated topical administration, similar effects were observed: small diffuse foci of small-droplet fatty degeneration of the liver, indications of parenchymatous swelling of the kidneys, and hemosiderin deposits in the spleen. Following inhalation, the following microscopic changes were observed: parenchymatous swelling of the liver, reduction of the white pulp of the spleen, fine deposits of hemosiderin in the lungs and accumulations of macrophages located subpleurally.

### **Study 4.6: Dermal Toxicity Study in Rats Treated with the Test Article Fomblin HC/25.**

**Study No:** 890778

**Volume and Page No.:** Vol. 2.17, page 030 [Ref. 5.4.22]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** July 19, 1989

**GLP Compliance:** Yes



**QA Report:** Yes (x) No ( )

**Methods and Dosing:** A single topical dose of 5000 mg/kg Fomblin HC/25 was administered to a clipped area ~6x5 cm on the dorsal area of the trunk (~10% TBS) and covered with an occlusive dressing for 24 hours.

**Species/Strain:** Sprague Dawley rat

**#/sex/group or time point:** 5 animals/sex

**Age:** 7-9 weeks

**Weight:** Males = 200-225 g; Females 175-200 g

**Supplier:** \_\_\_\_\_

**Dosage Groups in Administered Units:** 5000 mg/kg (30,000 mg/m<sup>2</sup>)

**Route, Form, Volume and/or Infusion Rate:** Topical, 2.63 ml/kg

**Drug Lot #, Radiolabel, and % Purity:** Batch no. S116 (11/10/87)

**Formulation/Vehicle:** Neat

**Observations and Times:** Animals were daily evaluated for 14 days post dosing.

**Clinical Signs:** Daily

**Body Weights:** Pre-Dosing and on days 8 and 15.

**Gross Pathology:** Necropsies were performed on all animals on day 14.

**Results:**

**Clinical Signs:** There were no mortalities or clinical signs of toxicity in any animal.

**Body Weights:** Weight gain during the observation period was within normal limits.

**Gross Pathology:** Gross examinations did not reveal any significant signs of local or systemic toxicity related to treatment.

**Study 4.7: Primary Dermal Irritation Study in New Zealand White Rabbits Treated with the Test Article Fomblin HC 25.**

**Study No:** 890759

**Volume and Page No.:** Vol. 2.17, page 051 [Ref. 5.4.22]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** August 8, 1989

**GLP Compliance:** Yes

**QA Report:** Yes (x) No ( )

**Methods and Dosing:** Acute topical doses of 0.5 ml Fomblin HC/25 were administered via patch (2.5 cm<sup>2</sup>) to intact and abraded clipped dorsal areas of the trunk and covered with an occlusive dressing for 24 hours.

**Species/Strain:** New Zealand White rabbit

**#/sex/group or time point:** 3 animals/sex

**Age:** 3 months

**Weight:** 2-3 kg

**Supplier:** \_\_\_\_\_

**Drug Lot #, Radiolabel, and % Purity:** Batch no. S116 (11/10/87)

**Formulation/Vehicle:** Neat

**Observations and Times:** Animals were examined on the two areas of intact skin and abraded skin for signs of erythema and edema at 24 and 72 hours and 7 days.

**Results:** There were no observed changes in behavior during the study period. Slight erythema on the abraded skin area was observed in one female at 24 and 72 hours. There were no other signs of irritation in any other animal at these time points or in any of the animals on day 7. Under the conditions of this study Fomblin HC/25 was considered non-irritating.

**Study 4.8: Acute Eye Irritation Study in New Zealand White Rabbits Treated with the Test Article Fomblin HC 25.**

**Study No:** 8900760

**Volume and Page No.:** Vol. 2.17, page 061 [Ref. 5.4.22]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** August 21, 1989

**GLP Compliance:** Yes

**QA Report:** Yes (x) No ( )

**Methods and Dosing:** Fomblin HC/25 (0.1 ml) was placed in the conjunctival sac of the right eye of each animal for a period of 24 hours.

**Species/Strain:** New Zealand White rabbit

**#/sex/group or time point:** 3 animals/sex

**Age:** 3 months

**Weight:** 2-3 kg

**Supplier:** \_\_\_\_\_

**Drug Lot #, Radiolabel, and % Purity:** Batch no. S116 (11/10/87)

**Formulation/Vehicle:** Neat

**Observations and Times:** Animals were examined at 1, 24 48 and 72 hours post-dosing and scored for irritation.

**Results:** There were no observed changes in behavior or discernable changes in treated eyes relative to the untreated eye at any evaluation time point. Under the conditions of this study Fomblin HC/25 was not considered to be an ocular irritant.

**Study 4.9: Fomblin HC/25 Comedogenicity Study in Rabbit Ear.**

**Study No:** 890761

**Volume and Page No.:** Vol. 2.17, page 106 [Ref. 5.4.24]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** November 14, 1989

**GLP Compliance:** Yes

**QA Report:** Yes (x) No ( )

**Methods and Dosing:** Fomblin HC/25 (1-ml/ear) was applied to the epidermal undersurface of the pinna in the concave area just external to the ear canal 24 hours/day for 14 consecutive days.

**Species/Strain:** New Zealand White rabbit

**#/sex/group or time point:** 6 males

**Age:** 2.5-3 months

**Weight:** 2.5-3.0 kg

**Supplier:** \_\_\_\_\_**Drug Lot #, Radiolabel, and % Purity:** Batch No. S116 (11/10/87)**Formulation/Vehicle:** Neat**Observations and Times:**

**Clinical Signs:** Animals were examined in the control and treatment sites for signs of erythema, edema, exfoliation, hyperplasia, folliculitis, necrosis and scab. Responses were scored at 24 hours after the beginning of the test and daily thereafter.

**Histopathology:** At the end of the treatment period all animals were euthanized and ~ 3 cm sections from treated and untreated sites were prepared for stereomicroscopic evaluation.

**Results:**

**Clinical Signs:** There were no mortalities or clinical signs of toxicity.

**Gross Pathology:** There were no significant gross differences noted between treated and untreated sites.

**Histopathology:** A slight increase in hyperkeratosis was seen in one rabbit in both the treated and untreated ears, and therefore not considered treatment related. There were no other visible changes in treated ears in relationship to untreated ears.

**Study 4.10: Two-Week Repeated dose Dermal Irritation Study in New Zealand White Rabbits  
Treated with the Test Article Fomblin HC/25®.**

**Study No:** 89B778**Volume and Page No.:** Vol. 2.17, page 073 [Ref. 5.4.23]**Conducting Laboratory and Location:** \_\_\_\_\_**Date of Study Initiation:** October 11, 1989**GLP Compliance:** Yes**QA Report:** Yes (x) No ( )

**Methods and Dosing:** Fomblin HC/25 (0.1 ml/day) was administered topically to the clipped thoracic area and 9% NaCl solution was administered on the abdominal areas for 14 consecutive days. On each animal, the right abdominal and thoracic areas of the skin were abraded at weekly intervals, while the left side areas were left intact. A gauze patch fixed to the skin with a porous tape was used to cover each application site and renewed every 24 hours with each new dose.

**Species/Strain:** New Zealand White rabbit**#/sex/group or time point:** 16 males, 6 females**Age:** 3 months**Weight:** 2-3 kg**Supplier:** \_\_\_\_\_**Drug Lot #, Radiolabel, and % Purity:** Batch no. S116 (11/10/87)**Formulation/Vehicle:** Neat**Observations and Times:****Clinical Signs:** Daily**Body Weights:** Pre-dosing and at termination.

**Gross Pathology:** Gross necropsies were performed on all animals.

**Histopathology:** x

**Results:**

**Clinical Signs:** There were no mortalities or clinical signs of toxicity during the study period.

**Body Weights:** Normal body weight gain was observed in all animals.

**Gross Pathology:** Focal ulcers and hemorrhagic areas in the stomach were observed in ~50% of the animals, their relationship to treatment is unknown. Erythema was noted at the abraded sites treated with 0.9 NaCl solution. There were no signs of irritation at the Fomblin HC/25 sites.

**Histopathology:** There were no signs microscopic lesions at the Fomblin HC/25 sites.

**Key Study Findings:** Under the conditions of this study, 14 day repeated topical applications of Fomblin HC/25 to intact and abraded skin produced no signs of local or systemic toxicity in rabbits.

**Study 4.11: Fomblin HC/25: Delayed Contact Hypersensitivity Study in Guinea-Pigs.**

**Report No:** — Report No. 87/MFL005/902

**Volume and Page No.:** Vol. 2.16, page 297 [Ref. 5.4.20]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** September 28, 1987

**GLP Compliance:** Yes

**QA Report:** Yes ( x ) No ( )

**Methods and Dosing:** The potential of Fomblin HC/25 to cause delayed contact hypersensitivity in guinea pigs was assessed by the maximization test developed by Magnusson and Kligman. An area of skin 4 x 6 cm overlying the scapula was clipped free of hair on the day prior to treatment.

**Induction:** The induction procedures were primary induction by intradermal injection on day 1 and secondary induction by occluded topical application on Day 8. Dermal responses to primary and secondary induction were assessed approximately 24 hours and 48 hours after injection or removal of the occlusive dressing.

Dorsal Medial Injection Site	Treatment	Control
Anterior	FCA	FCA
Middle	Fomblin HC/25	H <sub>2</sub> O
Posterior	Fomblin HC/25 + FCA	H <sub>2</sub> O + FCA

On day 7 10% sodium lauryl sulfate in petrolatum was applied to the clipped dorsa of all animals to enhance dermal absorption. On day 8, the dermal areas treated on day 1 were treated by topical application of 0.5 ml Fomblin HC/25 or 0.6 ml H<sub>2</sub>O. Each dose was absorbed onto a 4 x 2.5 cm absorbent patch which was applied to the skin and covered by an occlusive dressing for 48 hours.

**Challenge:** On day 22 each site was treated by topical application of 0.03 ml Fomblin HC/25. The dose was absorbed onto a 10 mm diameter absorbent patch which was covered by an occlusive dressing for approximately 24 hours.

**Species/Strain:** Albino, Dunkin-Hartley Guinea Pigs

**#/sex/group:** 5/sex/group

**Age:** ?

**Weight:** 321-462 g

**Supplier:** \_\_\_\_\_

**Drug Lot #, Radiolabel, and % Purity:** Batch S114

**Formulation/Vehicle:** Neat

**Observations and Times:** The induction and challenge sites were examined approximately 24 and 48 hours after removal of the occlusive dressings and the degree of reaction was scored on a five point scale from no response to severe erythema. Body weights were also recorded weekly to detect treatment-related effects.

**Results:**

**Body Weight:** Bodyweight gains were normal throughout study period.

**Primary Irritation Evaluations:** Intradermal injection of Fomblin HC/25 caused barely perceptible erythema. Injection of test material in combination with FCA caused slight or moderate erythema. Occluded topical applications caused no reaction.

**Sensitization Evaluations:** Challenge application of Fomblin HC/25 caused no dermal reaction.

**Study 4.12: Contact Photosensitivity Study in Guinea-Pigs.**

**Study No:** —Draft Report 88/0355

**Volume and Page No.:** Vol. 2.17, page 001 [5.4.21]

**Conducting Laboratory and Location:** \_\_\_\_\_

**Date of Study Initiation:** March 8, 1988

**GLP Compliance:** Yes

**QA Report:** Yes ( ) No (x)

**Methods and Dosing:** The potential of Fomblin HC/25 to cause photosensitization in guinea pigs was assessed on area of clipped skin ~4 x 6 cm overlying the scapula. UVR source was an array of \_\_\_\_\_ fluorescent tubes emitting UVA and UVB wavebands.

**Induction:** On day 1, all animals were subject to a single intradermal injection of 0.1 ml of Freund's Complete Adjuvant (FCA) into the dorsum overlying the scapula. Animals were irradiated following topical application of 0.1 ml Fomblin HC/25 or 25% w/v TSCA (positive control) on days 1, 2, 5 and every second day until day 21. After a 20 minute period for absorption, sites were exposed to UVR for ~20 minutes. The negative control group remained untreated during the induction phase.

**Challenge:** On day 35 four challenge sites were identified and treated by topical application of 0.05 ml of the appropriate solution. Two of the sites were shielded and the other two were exposed to UVR.

**Species/Strain:** Albino, Dunkin-Hartley Guinea Pigs

**#/sex/group or time point:** 5/sex/group

Age: ?

Weight: 240-308 g

Supplier: \_\_\_\_\_

Drug Lot #, Radiolabel, and % Purity: Batch S114

Formulation/Vehicle: Neat

**Observations and Times:**

**Primary Irritation Evaluations:** Dermal reactions to the induction procedures were assessed approximately 24 hours after each application.

**Sensitization Evaluations:** challenge reactions were assessed at 24, 48 and 72 hours after dosing.

**Results:**

**Primary Irritation Evaluations:** Topical application of Fomblin HC/25 and 25% w/v TSCA to positive control animals, followed by UV irradiation, caused slight or moderate erythema, exfoliation and eschar formation during the eleven induction phases.

**Sensitization Evaluations:** Only one animal treated with Fomblin HC/25 displayed slight erythema at the irradiated site 24 hours after dosing. All other animals and time-points were negative. Controls performed within historic limits.

**Key Study Findings:** Under the conditions of this study, Fomblin HC/25 did not cause contact photosensitivity in guinea pigs.

## **V: GENOTOXICITY AND CARCINOGENICITY STUDIES**

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**Genotoxicity:** Nonclinical information was not submitted to assess the genotoxic potential ICD 2289. However, Fomblin HC/25 was negative when at concentrations up to 5000 µg/plate using the standard Ames Assay in *Salmonella typhimurium*.

**Carcinogenicity:** The carcinogenicity potential of ICD 2289 or its components has not been studied.

## **VI: REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY**

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Ref. 5.4.25: Successful Pregnancy Outcome Following Mid-gestational Uterine Rupture and Repair Using Gore-Tex Soft Tissue Patch. (1990) Obstet Gynecol 75(3, Pt. 2):518-521). There were no reported adverse fetal or maternal effects following surgical insertion of an expanded polytetrafluoroethylene Gore-Ex soft tissue patch to assist uterine integrity following the 19<sup>th</sup> week of gestation.

This report is not relevant to the small molecular weight polytetrafluoroethylene particles used in TSP. No other relevant nonclinical information was not submitted to assess the reproductive and developmental toxicity potential ICD 2289 or its components.

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**Overall Summary of Toxicity Studies Performed with ICD 2289 or Fomblin HC/25**

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**Oral Toxicity:** There were no adverse effects noted following the oral administration of up to 3240 mg/kg (19,440 mg/m<sup>2</sup>) ICD 2289, the highest dose tested orally in rats. The equivalent dose in man would be approximately 540 mg/kg (19,980 mg/m<sup>2</sup>) or approximately 32 g for an average adult weighing 60 kg. Accidental ingestion of this magnitude is unlikely in humans, however, a single pouch of ICD 2289 contains 84 g TSP or approximately 51.8 g/m<sup>2</sup> for an average 60 kg adult.

**Skin Irritation – Intact and Abraded Skin:** ICD 2289 is not considered a primary skin irritant in rabbits following applications to either intact or abraded skin. Repeated applications of 2000 mg/kg/day (24.0 g/m<sup>2</sup>/day) ICD 2289 to the skin surface of rabbits for 21 consecutive days did not cause any significant signs of systemic toxicity. Dermal signs of toxicity consisted of mild, subacute inflammation. (*Maximum potential human exposure = 51.8 g/m<sup>2</sup>*)

**Eye Irritation:** Instillation of ICD 2289 or Fomblin HC/25 in rabbit eyes for 24 hours did not result in any adverse ocular effects.

**Skin Sensitization:** ICD 2289 was not considered to be a sensitizer in guinea pigs.

**Photochemical Skin Irritation:** In guinea pigs, there were no signs of ICD 2289 –induced photochemical skin irritation. Having no double bonds, ICD 2289 would not be expected to absorb light in the UVB, UVA or visible wavelengths and therefore would not be expected to be photochemically reactive.

**Fomblin HC/25:** In addition to the studies performed with ICD 2289, a number of nonclinical studies were also submitted demonstrating the safety of Fomblin HC/25. Briefly, there were no signs of local or systemic toxicity following acute doses of up to 15 g/kg administered orally and 5 g/kg administered dermally and by ip in rats. Following a 4-week oral study in rats, the only remarkable finding was related to microscopic findings in the kidney tubules of male rats; 4/5 test males showed some basophilic and/or dilated tubules compared to 0/5 controls. Acute and repeated topical applications of Fomblin HC/25 did not result in any significant signs of dermal irritation in rabbits, comedogenicity in rabbit ears, or contact sensitization or photosensitizing potential in guinea pigs.

Fomblin HC/25 was negative when at concentrations up to 5000 µg/plate using the standard Ames Assay in *Salmonella typhimurium*.

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**COMPREHENSIVE SUMMARY OF NONCLINICAL DATA**

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Percutaneous exposure to small quantities ( $\text{mg}/\text{m}^2$ ) of chemical warfare agents can result in extremely deleterious or even lethal effects. Development of a topical skin protectant is to provide an integrated approach to improving overall effectiveness in the field. Optimally, this agent should enhance the soldier's ability to both survive and operate in hazardous environments with maximal effectiveness.

**Pharmacology and Pharmacokinetics:** ICD 2289, here after referred to as TSP, acts by providing a physical barrier to prevent dermal contact from a wide variety of chemical warfare agents having very different chemical properties. TSP has no other known pharmacologic action other than to serve as an antipenetrant barrier at the surface of the skin where it is applied. Intact stratum corneum appears to act as an effective barrier against penetration and subsequent absorption of TSP following dermal application.

**Nonclinical Efficacy Data:**

**In Vitro Efficacy Studies:** *In vitro* studies were conducted utilizing M8 Chemical Agent Detector Paper which is used by the army to detect chemical warfare agents by changing color upon contact with a variety of CWAs. Pretreatment of C8 paper with a layer of TSP approximately 0.15 mm in thickness one hour prior to challenge with TGD and HD was shown to effectively act as an antipenetration barrier for the up to 6 hours.

CWA	w/TSP	w/o TSP
HD	>6 hours	Immediate
TGD	>6 hours	Immediate

The time interval between application of TSP, i.e., wear-time, and subsequent challenge with HD was also evaluated for up to 24 hours. Time intervals between 5 minutes and 24 hours between application of TSP and HD did not appear to have any adverse effect on the *in vitro* performance of TSP. However, this test did not account for the effects of body heat, sweat, or friction caused by clothing on TSP 'wearability'. Unfortunately, *in vivo* nonclinical studies were also not designed to simulate human usage. Nonclinical exposures were typically conducted on anesthetized animals for a maximum of 4 hours, and CWA challenges were performed 1 hour after TSP applications.

Concurrent *in vitro* applications of the insecticide permethrin, up to a concentration of  $6.25 \mu\text{g}/\text{cm}^2$ , and military issue sunscreens did not appear to interfere with effectiveness of TSP when challenged with HD. However, when permethrin was applied at  $62.5 \mu\text{g}/\text{cm}^2$ , two (2/9) breakthroughs occurred within the first hour after HD challenge, indicative that permethrin may have altered the antipenetrant effects of TSP.

**In Vivo Efficacy in Animals Models:** CWA concentrations used in nonclinical studies to evaluate the antipenetrant effects of TSP were normalized across species by the use of the LD<sub>50</sub>. However, in all instances, the concentrations chosen were equal to or greater than concentrations expected on the battlefield. Percutaneous LD<sub>50</sub> doses of nerve agents, extracted from the literature for man (~70 kg) are as follows:

GD	1.4 mg/kg
VX	0.14 mg/kg



Rabbits were chosen as the most sensitive laboratory model for CWAs, but also because they have demonstrated a similar pattern as man to both organophosphate poisoning, i.e., lacrimation, local fasciculations, tremors, convulsions and death, and local irritation to HD induced skin injury, i.e., frank blistering and necrotic histopathology. Concentrations of GD and VX evaluated in rabbits were as follows:

TGD	3.35 mg/kg	( 1 x Rabbit LD <sub>50</sub> )
GD	8.96 mg/kg	( 1 x Rabbit LD <sub>80</sub> )
VX	0.50 mg/kg	(10 x Rabbit LD <sub>50</sub> )

Multiple biological endpoints were used to evaluate the effectiveness of TSP following topical exposures of HD in guinea pigs and HD, GD, TGD, VX and T-2 in rabbits. Endpoints evaluated included measurements of RBC and plasma AChE activity and lethality for the nerve agents, and dermal irritation for HD and T-2.

In anesthetized rabbits, applications of TSP prior to a 4-hour challenge with 1  $\mu$ l liquid HD significantly reduced both the size of the lesions and the mean lesion area ratio (LAR) when compared to pretreatment with PEG 540. Mean lesion sizes at 24 hours were 3.5 to 5 fold smaller than lesions at unprotected sites and the mean LAR was reduced by 70 to 80 % (Studies 2.6, 2.8 2.9 and 2.14). Pretreatment with TSP prior to a 3-hour challenge with HD vapor (1.4 mg/L) in guinea pigs, was also shown to significantly reduce erythema by 43% (Study 2.7) over a 4 hour treatment/observation period.

Pretreatment with TSP was also shown to effectively block all macroscopic signs of dermal irritation 24 to 48 hours following a 6-hour challenge with T-2 toxin (Study 2.15). Histopathological findings were also significantly ( $p < 0.05$ ) diminished from moderate to marked lesions in controls to only minimal when present at TSP protected sites. Histological lesions from the TSP pretreated sites consisted of superficial dermatitis, edema, folliculitis, and epidermal necrosis; and intradermal pustules in 1/8 sites. Unprotected sites also presented with moderate to marked deep dermatitis, panniculitis, dermal necrosis and intradermal pustules at 8/8 sites.

Following a 15 minute challenge of 0.1 ml of 1.0% CS/TOF with and without TSP, signs of erythema were very mild and first appeared 15-60 minutes after exposure. Edema was observed only after 24 hours. Test sites protected by TSP demonstrated significantly milder reactions (barely perceptible) when compared to reactions at unprotected sites (Study 2.16). At 4 and 24 hours post challenge, all unprotected sites (20/20) evinced erythema and edema whereas only mild erythema was observed in 6/20 animals in the TSP treatment group at 4 hours and 9/20 animals (1 with edema) at 24 hours.

*In vivo* studies designed to assess penetration of nerve agents based on AChE activity demonstrated significant protection relative to the unprotected animals against 4-hour challenges with TGD, GD and VX. However, almost 100% of the animals exposed to TGD and GD, and approximately 67% of the animals exposed to VX lost some AChE activity during the 4-hour challenge period, indicative of penetration of the TSP barrier.

In terms of mean RBC and whole blood AChE activity, TSP demonstrated significant protection relative to unprotected animals against 4-hour challenges with lethal doses of TGD, GD and VX. Exposures to GD resulted in some reduction in whole blood AChE activity in over 99% of the animals (145/146) indicative of some penetration of the TSP barrier, whereas exposures to VX

resulted in between 10 and 33% of the animals with no detectable changes in AChE activity over the 4 hour challenge period.

In terms of lethality, TSP was effective in significantly reducing the lethal effects of GD by over 90% and VX by 60-90% following 4 hour challenges. In unprotected animals, 100% mortality was observed within minutes of challenge. Lethality appeared to be preceded by complete loss of AChE activity.

Effects of CWAs on RBC AChE Activity and Mortality (Study 2.12):

Parameter	Time (h)	TGD		VX	
		w/o TSP	w/TSP	w/o TSP	w/TSP
Mean AChE Levels (U/ml)	4	0.2 ± 0.2	1.2 ± 0.4	0.0 ± 0.0	1.1 ± 0.8
Mean AChE Activity (%)	4	10 ± 10	60 ± 20	0 ± 0	50 ± 40
% Animals w/100% AChE Activity	4	0 %	0 %	0 %	8 %
% Animals w/<50% AChE Activity	4	100 %	35 %	100 %	46 %
% Animals w/<10% AChE Activity	4	92 %	4 %	100 %	29 %
% Animals w/<0% AChE Activity	4	8 %	4 %	100 %	4 %
Mortality Rate	4	8 %	4 %	100 %	4 %
	24	12 %	8 %	100 %	8 %

Effects of CWAs on Whole Blood AChE Activity and Mortality (Studies 2.13).

Parameter	Time (h)	GD		VX	
		w/o TSP	w/TSP	w/o TSP	w/TSP
Mean AChE Levels (U/ml)	4	0.0 ± 0.1	0.4 ± 0.3	0 ± 0	0.6 ± 0.6
Mean AChE Activity (%)	4	0	30 ± 20 %	0	50 ± 40 %
No. of Animals w/100% AChE Activity	4	0	<1.0 %	0	33 %
No. of Animals w/<50% AChE Activity	4	100 %	75 %	100 %	54 %
No. of Animals w/<10% AChE Activity	4	100 %	26 %	100 %	42 %
No. of Animals w/<0% AChE Activity	4	100 %	7 %	100 %	34 %
Mortality Rate	4	100 %	7 %	100 %	35 %
	24	100 %	9 %	100 %	39 %

**Effect of Other Topical Military Products on TSP:** There were notable decreases observed in the protective effects of TSP when used in conjunction with some types of camouflage paints (loam and sand) and insect repellents containing DEET or permethrin. Optimally these products should not be used concurrently, and soldiers should be instructed not to apply these agents either prior to or following TSP application. However, if the agent has already been applied, soldiers should still be encouraged to apply TSP after removing as much of the agent as possible with a dry cloth.

The effect of sun screens was not evaluated *in vivo*.

**Nonclinical Toxicity Data:** Following oral administration of TSP to rats, there were no signs of adverse effects noted following doses up to 3240 mg/kg (19.4 g/m<sup>2</sup>) TSP, the highest dose tested. The equivalent dose in man would be approximately 540 mg/kg (20 g/m<sup>2</sup>) or approximately 32 g for

an average adult weighing 60 kg. A single pouch of TSP contains 84 g TSP or approximately 51.8 g/m<sup>2</sup> for an average 60 kg adult. In a 4-week oral study in rats with Fomblin HC/25, the only remarkable finding was related to microscopic findings in the kidney tubules of male rats; 4/5 test males showed some basophilic and/or dilated tubules compared to 0/5 controls.

TSP is not considered a primary ocular or dermal irritant in rabbits. Furthermore, repeated applications of 2000 mg/kg/day (24.0 g/m<sup>2</sup>/day) TSP to the intact or abraded skin of rabbits for 21 consecutive days resulted in only mild signs of subacute dermal inflammation, and no significant signs of systemic toxicity. When tested for skin sensitization and phototoxicity potential in guinea pigs, TSP was not considered to be a potential sensitizer or photo toxicant.

*Carcinogenesis, Mutagenesis, and Impairment of Fertility:* The genotoxicity and carcinogenicity potential of TSP were not evaluated. However, Fomblin HC/25 (referred to as PFPE in the label) was negative when tested at concentrations up to 5000 µg/plate using the standard Ames Assay in *Salmonella typhimurium*. Nonclinical information was not submitted to assess the potential effect of TSP on fertility.

*Usage in Pregnancy:* Relevant nonclinical information was not submitted to assess the reproductive and developmental toxicity potential TSP or its components.

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## DISCUSSION

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Points to consider in using animal efficacy studies as surrogates for human clinical studies (Federal Register, 10/5/99, Vol 64, No. 192):

- 1) *There is a reasonably well understood pathophysiological mechanism for the toxicity of the chemical, biological, radiological, or nuclear substance and its amelioration or prevention by the product;*

CWA concentrations used in the nonclinical studies to evaluate the antipenetrant effects of TSP were normalized across species by the use of the LD<sub>50</sub>. However, in all instances, the concentrations chosen were equal to or greater than concentrations which the Army predicts might be encountered under battlefield conditions.

- 2) *the effect is independently substantiated in multiple animal species, including species expected to react with a response predictive for humans;*

Although only rabbits were used for the primary pivotal studies, they appear to be one of the most sensitive laboratory models for CWAs based on skin permeability and LD<sub>50</sub> values to the representative CWAs used for testing. They also demonstrated a pattern similar to man to both organophosphate poisoning, i.e., lacrimation, local fasciculations, tremors, convulsions and death, and to HD-induced skin injury, i.e., frank blistering and necrotic histopathology.

- 3) *the animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity;*

The use of lesion area ratios, inhibition of AChE activity, and mortality are relevant to CWA exposures in humans. However, due to possible species differences on the relationship of RBC or whole blood AChE with suppression of AChE activity in critical organ tissues such as brain, heart, lungs and kidneys, the data should only be used to indicate the degree to which nerve agents were able to penetrate the TSP barrier.

- 4) *the data or information on the kinetics and pharmacodynamics of the product or other relevant data or information in animals and humans allows selection of an effective dose in humans and it is therefore reasonable to expect the effect of the product in animals to be a reliable indicator of its efficacy in humans.*

Unfortunately it was not possible to test TSP in animals under conditions similar for use in humans. For humane reasons and to prevent disruption of the application sites by animal movement, it was necessary to anesthetize the animals throughout the 4-hour exposure periods. Therefore, factors such as prolonged wear time, changes in temperature, sweat, and friction caused by clothing could not be evaluated in animals.

Given the limitations in extrapolating animal exposures to potential human exposures in combat situations, the Sponsor appeared to adequately demonstrate the following points:

- TSP significantly reduced the size and histology of lesions resulting from 4-hour topical exposures to HD, T-2 toxin and CD.
- AChE appeared to be a sensitive marker for nerve agent penetration of the TSP barrier.
- There was a good correlation between complete loss of either RBC or whole blood AChE activity and mortality.
- Compared to untreated animals, TSP provided protection against loss of AChE activity in a significant number of animals subjected to 4-hour CWA exposures.
- Although the effects of TSP were reduced by the use of certain camouflage paints and insect repellents containing DEET or permethrin following challenges with HD and VX, significant protective effects (~50%) were maintained when compared to unprotected animals.
- Protection in animals was dependent on complete coverage of exposed sites.

As previously discussed, there were several points which were not adequately evaluated in the nonclinical studies. These included 1) the optimal or minimal time between application of TSP and exposure to CWAs, and 2) the maximum efficacy time for TSP following application. *In vivo* animal studies were primarily conducted utilizing a 1-hour time interval between TSP applications and CWA challenge. Only two *in vivo* studies were conducted utilizing a 15-minute interval between TSP application and CWA challenge and no studies were conducted with intervals of greater than 1 hour. Study 3.7 was conducted in guinea pigs with 5, 10, 15 and 20 minute challenges with HD vapor. This study demonstrated no protective effect following the 15- and 20-minute exposures. In Study 3.16, TSP was shown to provide significant protection to rabbits exposed to CS/CR for 15 minutes. With only one exception, *in vivo* animal exposures were conducted for a maximum of 4 hours. In study 3.15, rabbits were exposed to T-2 toxin for 6 hours with no loss of protectivity. It would have been beneficial to evaluate nerve agent exposures greater than 4 hours to determine a recommended maximum exposure time.

In addition to inadequate evaluation of times involving TSP application and CWA exposure, the effects of factors such as temperature, humidity, sweat, and friction from skin and clothing were not evaluated in the animal studies.

Finally, TSP applications in animals were made using a uniform thickness applied using a spatula and exposure wells. The efficacy produced in animals was dependent on careful application and complete coverage of the exposed area. Even small breaks in the coverage resulted in significant morbidity and lethality. However, the fact that soldiers should be using TSP only in conjunction with protective clothing should help to diminish the effects of uneven applications in the field.

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ON ORIGINAL

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**RECOMMENDED LABELING CHANGES**

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Recommended labeling changes relevant to nonclinical data are presented below. The Sponsor's submitted labeling sections (*in italics*) relevant to nonclinical data are followed by the reviewer's recommendations.

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WITHHOLD 3 PAGES

Draft

Labeling

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**CONCLUSION**

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From a Pharmacology/Toxicology perspective, this NDA can be approved, provided that labeling is appropriately revised. TSP significantly reduced the effects in rabbits of 4-hour topical exposures of the chemical warfare agents HD, T-2 Toxin, GD and VX. It appears safe, with no detectable dermal absorption, or local or systemic effects. The genotoxic and carcinogenic potential, and effects on reproduction and development have not been evaluated.

Due to the possible adverse effects associated with smoking cigarettes contaminated with TSP, it is recommended that the Sponsor perform a chronic nonclinical study to evaluate long-term effects of exposures to polytetrafluoroethylene fumes.

/S/

1-28-2000

Lynnda Reid, Ph.D.  
Pharmacologist/Toxicologist

Date

cc:

~~IND~~ IND  
HFD-540

HFD-540/Pharm/Reid

HFD-540/Pharm/Jacobs

HFD-540/CSO/Cross

HFD-540/MO/Okun

HFD-540/Chem/Timmer

HFD-540/BioPharm/Bashaw

For Concurrence Only:

HFD-540/DD/JWilkin

HFD-540/TL/AJacobs /S/ 1-28-00



FEB 25 2000

## REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Key Words: Topical Skin Protectant

Reviewer: Lynnda Reid, Ph.D.

Division: Dermatologic and Dental Drug Products, HFD-540

Date: February 18, 2000

NDA No: 21-084

Dates: Faxed data received February 17, 2000

Sponsor: Office of the Surgeon General  
 Department of the Army  
 Commander, U.S. Army Medical Research and Materiel Command  
 Attn: MCMR-RCQ  
 Fort Detrick, Frederick, MD 21702-5012  
 (301) 619-2165

Drug: Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA)  
 (Formerly called TSP and ICD 2289)

## 1) PTFE (50%): Polymist® F5A

Chemical Name(s): Polytetrafluoroethylene; Polytef; Ethene, tetrafluoro, homopolymer

CAS Number: CAS-9002-84-0

Molecular Formula:  $(CF_2CF_2)_n$  - Polymer of recurring tetrafluoroethylene units

Description: Fine White Particulate

## 2) PFPE (50%): Fomblin Y 25® or Fomblin HC/25

Chemical Name(s): Perfluoroalkylpolyether; Trifluoromethyl-poly [oxy-2-(3-trifluoromethyl)-trifluoro-ethylene]-poly(oxy-difluoromethylene)-trifluoromethoxy; propene 1,1,2,3,3,3-hexafluoro, oxidized, polymerized; perfluorinated polyether; perfluoropolyether; polyoxyperfluoroalkane; perfluoropolymethylisopropyl ether.

CAS Number: CAS 69991-67-9

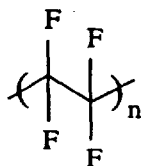
Molecular Formula:  $C_xF_{2x+1}-(O-CF(CF_3)-CF_2)_n-(O-CF_2)_m-O-C_yF_{2y+1}$   
 where x, y = 1, 2 or 3 and n/m > 40.

Average Molecular Weight: 3200;

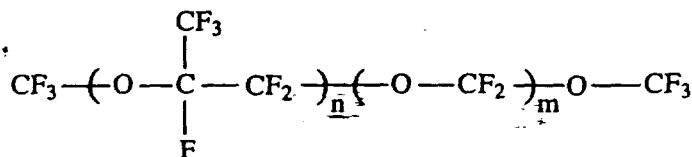
Average Viscosity: 250 cSt

Description: Colorless, Odorless Oil

Chemical Structure(s):



PTFE



PFPE

**Clinical Formulation and Route of Administration:** SERPACWA is composed of polytetrafluoroethylene and perfluoroalkylpolyether and (PTEE/ PFPE 1:1). Physically, the substance is a heavy white cream, greasy to the touch. It is intended for topical administration and will be supplied in a single use 84 g/unit pouch.

**Drug Class:** Topical Barrier Cream

**Indication:** For the prevention of percutaneous penetration and subsequent toxicity of chemical warfare agents.

**Related Documents:** IND —

**Directions for Use:** SERPACWA is indicated for protection of the skin from contact with chemical warfare agents (CWA). It is to be used in conjunction with appropriate chemical protective clothing and applied prior to exposure to CWA. Military personnel will be instructed in the use of SERPACWA during training. Before applying the chemical protective overgarment, sweat, insect repellent, sand or dirt should be wiped from the skin with a dry towel. Approximately 1/3 of the packet should be rubbed evenly around the wrists, neck and boot tops of lower legs forming a barely noticeable white film. The remaining 2/3 should be rubbed evenly onto armpits, groin area and waistline. To remove SERPACWA, scrub sites with a dry towel or cloth using soap and water.

## INTRODUCTION

Revised labeling was received via FAX after 6:00 p.m. on February 16, 2000. The newly proposed labeling contained the following statement:

[ ]

On February 17, 2000, the Sponsor was asked to submit the nonclinical information referenced to support this statement. The 60 page report for Pre-Task Pilot Study 91-14 entitled "The Effect of A Candidate Topical Skin Protectant — on the Efficacy of the M291 Skin Decontamination Kit Against TGD and HD in the Rabbit", was received by FAX just prior to the final labeling meeting. These data were used to support the following proposed labeling:

"The potential for interaction between SERPACWA and the — Skin Decontamination Kit has not been characterized — However, in animals, the protection provided by a SERPACWA-like product and a skin decontamination kit was superior to that provided by the skin decontamination kit alone."

A second FAX was also submitted regarding the use of permethrin impregnated battle dress uniforms (BDU) as it relates to potential interactions between permethrin and SERPACWA. This review contains a complete review of the materials faxed on February 17, 2000.

**Disclaimer:** Note some material may be taken directly from Sponsor's submission.

## I Review of Pre-Task Pilot Study 91-14:

**Study Title:** The Effect of A Candidate Topical Skin Protectant \_\_\_\_\_ on the Efficacy of the M291 Skin Decontamination Kit Against TGD and HD in the Rabbit

**Study No:** Pre-Task Pilot Study 91-14, Contract DAMD17-89-C-9050

**Conducting Laboratory and Location:** Battelle Columbus Operations, 505, King Avenue, Columbus, OH

**Date of Study Report:** August, 1991

**Methods/Dosing:** The prototype to SERPACWA used in this study was identified as \_\_\_\_\_. This compound is composed of \_\_\_\_\_ PTFE and \_\_\_\_\_ PFPE in comparison to SERPACWA (ICD2289) which has a 50:50 composition of PTFE and PFPE. The objective of this study was to determine whether a 0.1 mm-thick layer of \_\_\_\_\_ interfered with the ability of M291 Skin Decontamination Kit (SKD) to decontaminate either TGD or HD exposures in male New Zealand White rabbits (2-4 kg). The methods described in MREF Protocol 68 were followed in performing these studies.

For evaluation against HD challenge, exposures were fixed at 1.0  $\mu$ l per application site and the relative lesion areas from sites pretreated with \_\_\_\_\_ were compared with those of sites not pretreated with \_\_\_\_\_ to determine the effect of the topical skin protectant on the ability of the M291 SKD to provide protection against HD exposure when decontamination was initiated 1, 3 or 5 minutes following HD application. Seven sites were marked on the clipped dorsum of each rabbit. \_\_\_\_\_ was applied to 3 of these sites. Approximately 1 hour later, 1  $\mu$ l of liquid HD was applied to all 7 sites. Two test sites, one with \_\_\_\_\_ pretreatment and one without, were decontaminated with M291 SDK according to standard decontamination procedures at 1, 3 and 5 minutes following HD exposure. The 7<sup>th</sup> site, which was not decontaminated nor pretreated with \_\_\_\_\_, served as a control site and was used as a basis for calculation of lesion area ratios (LARs). A total of 24 male rabbits were challenged, 8/day on three consecutive test days.

The impact of \_\_\_\_\_ pretreatment on the ability of M291 SDK to decontaminate skin exposed to thickened soman (TGD) was determined from the dose of TGD required to decrease rabbit erythrocyte acetylcholinesterase (AChE) by 50% (ID<sub>50</sub>) in control and M291 SDK-decontaminated animals both with and without \_\_\_\_\_ treatment. Blood samples were drawn via indwelling catheter from an ear artery prior to \_\_\_\_\_ pretreatment, approximately 60 minutes after \_\_\_\_\_ pretreatment (just prior to TGD challenge), and at 30, 60 and 120 minutes following TGD exposure. Decontamination with M291 SDK was initiated 2 minutes following TGD applications. The dose site was decontaminated by dabbing the exposed area for a 10 second period, at the end of which, the area was wiped once. TGD doses ranged between 0.1 and 33.5 mg/kg with 4 to 8 rabbits tested at each dose with a total of 120 rabbits tested.

**Results:** The combined use of \_\_\_\_\_ and the M291 Skin Decontamination Kit significantly increased the concentration of TGD necessary to cause a 50% reduction in AChE activity (ID<sub>50</sub>) when compared to unprotected skin and to unprotected skin decontaminated with the M291 SDK (Table 1). It can also be seen that the lesion area ratios (LAR) were significantly reduced in rabbits when exposure sites were pretreated with \_\_\_\_\_ and decontaminated with the M291 SDK (Table 2).

Table 1: LD<sub>50</sub> following M291 SDK decontamination of non-pretreated and — pretreated test sites following 30, 60 and 120 minutes after TGD challenges (n=20).

Time to Decontamination	TGD ID <sub>50</sub> (mg/kg)		
	TGD	TGD + M291	— + TGD + M291
30 minutes	1.24 – 2.52	1.93 – 3.14	40.0 – 103
60 minutes	0.64 – 1.14	1.44 – 2.41	27.9 – 52.6
120 minutes	0.52 – 0.88	1.14 – 2.05	19.7 – 34.0

Table 2: Lesion area ratios following M291 SDK decontamination of non-pretreated and — pretreated test sites following 1, 3 and 5 minutes after HD challenges (n=23).

Time to Decontamination	LAR	
	HD + M291	— + HD + M291
1 minute	0.178 ± 0.134	0.021 ± 0.031
3 minutes	0.237 ± 0.099	0.032 ± 0.029
5 minutes	0.343 ± 0.173	0.061 ± 0.075

This data clearly demonstrates that the use of — in conjunction with M291 Skin Decontamination Kit is superior to the use of decontamination alone in protecting animals against TGD-induced loss of AChE and HD-induced skin lesions.

It would have been useful if the study had also included sites which were pretreated with — and challenged with CWA but not decontaminated to determine if decontamination had any adverse effect on the barrier properties of —

**Key Study Finding:** The protection provided by —, a SERPACWA-like product, and the M291 Skin Decontamination Kit was superior to that provided by the skin decontamination kit alone.

## II Addendum to M8 Paper Test – Study 3.3 of the Original NDA 21-084 Review (N000):

This purpose of this addendum report is to explain the application rates of permethrin used in the referenced *in vitro* study and to make a correction. The following two reference articles were also submitted:

- 1) Snodgrass HL (1992) Permethrin Transfer from Treated Cloth to the Skin Surface: Potential for Exposures in Humans. J of Toxicol and Environ Health 35:91-105.

2) Insect/Arthropod Repellent: Protective Treatment for Military Battle Dress Uniform (1989)  
EPA Registration Supplement, Volume 3, Permethrin Quantitative Risk Assessment.

Briefly, the original study report stated that 25  $\mu$ l aliquots of a solution containing 19.6 or 196 mg of permethrin in 25 ml of isopropanol were applied onto ICD 2289 pretreated M8 Chemical Detection Paper and allowed to dry prior to application of 8  $\mu$ l liquid sulfur mustard (HD). The target permethrin application rates were 6.25 and 62.5  $\mu$ g/cm<sup>2</sup>. After 360 minutes, there were no breakthroughs in paper treated with ICD 2289 alone or with ICD 2289 plus 6.25  $\mu$ g/cm<sup>2</sup> permethrin. However, there were 2 HD breakthroughs (2/9) with ICD 2289 plus 62.5  $\mu$ g/cm<sup>2</sup> permethrin, occurring 25 and 42 minutes after HD applications.

**Selection of Permethrin Application Rates:** The application rates of 6.25 and 62.5  $\mu$ g/cm<sup>2</sup> were selected to represent a 10 and 100 fold excess of the maximum anticipated transfer rate of permethrin from impregnated clothing to skin. The transfer rate of <sup>14</sup>C-labeled permethrin from cloth impregnated with 0.125 mg/cm<sup>2</sup> permethrin to rabbit skin was reported to be 0.5% per day (Article 1) or 0.62  $\mu$ g/cm<sup>2</sup>/day. The army predicted exposure rates of approximately 0.005 mg/kg/day (equivalent to 0.185 mg/cm<sup>2</sup>/day for an average 60 kg adult) based on continuous wear of a uniform impregnated with permethrin at a target concentration of 0.125 mg/cm<sup>2</sup> (Article 2).

**Study Report Correction:** The original study report stated that both the 6.25 and 62.5  $\mu$ g/cm<sup>2</sup> doses were applied prior to ICD 2289. This was incorrect. The 6.25  $\mu$ g/cm<sup>2</sup> dose was applied prior to ICD 2289 as stated, however, the 62.5  $\mu$ g/cm<sup>2</sup> dose was applied after ICD 2289.

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## SUMMARY AND CONCLUSION

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1) Study 91-14 supports the following proposed labeling:

The potential for interaction between SERPACWA and the Skin Decontamination Kit has not been characterized. However, in animals, the protection provided by a SERPACWA-like product and a skin decontamination kit was superior to that provided by the skin decontamination kit alone.

2) Potential interactions between SERPACWA and permethrin:

The *in vitro* study performed to evaluate possible interactions between permethrin and ICD 2289 was inconclusive. The exposure multiples are based on estimates of the transfer of permethrin from clothing impregnated with a constant concentration of 0.125 mg/cm<sup>2</sup> permethrin at a rate of 0.5% per day based on studies in rabbits. However, at the present time, soldiers are not being issued permethrin impregnated clothing, but instead 0.5% aerosolized permethrin in 6 oz cans is being distributed for direct application by personnel to uniforms. Furthermore, although the ICD 2289 barrier may have been disturbed while applying the 62.5  $\mu$ g/cm<sup>2</sup> dose as the Sponsor has hypothesized, follow up studies were not performed to rule out a possible interaction between high concentrations of permethrin

and/or its solvent, isopropanol, and ICD 1189. In addition, because the procedures for applying the low and high doses of permethrin were not the same, a dose-response correlation can no longer be made. Since high concentrations of permethrin could occur following direct applications onto skin prior to, or after SERPACWA application, the labeling sections indicating a possible adverse effect by permethrin on the barrier properties of SERPACWA should remain in the labeling.

/S/

Lynnda Reid, Ph.D.  
Pharmacologist/Toxicologist

2-24-00

Date

cc:

NDA 21-084

HFD-540

HFD-540/Pharm/Reid

HFD-540/Pharm/Jacobs

HFD-540/CSO/Cross

HFD-540/MO/Okun

HFD-540/Chem/Timmer

HFD-540/BioPharm/Bashaw

For Concurrence Only:

HFD-540/DD/JWilkin

HFD-540/TL/AJacobs

/S/ 2/24/00 DFS ✓  
/S/ 2/24/00 HFD

APPEARS THIS WAY  
ON ORIGINAL